AMENDMENTS TO THE CLAIMS

The following is a complete listing of all claims, with markings to show amendments and status identifiers in accordance with 37 C.F.R. §1.121(c). Currently amended claims are marked showing deletions with ~~strikethrough~~ or enclosure in double brackets [[ ]], and showing added text by underlining. This listing of claims will replace all prior versions and listings of the claims in the application.

1. (PREVIOUSLY PRESENTED) A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature, T_1, for denaturation of the strands of the target nucleic acids or their primer extension products, and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2, and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3, provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same,

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic polymeric volume exclusion agent.

2. (PREVIOUSLY PRESENTED) A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature, T_1, for denaturation of the strands of the target nucleic acids or their primer extension products, and

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2, and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3, provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same,

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, a DNA polymerase and a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridization, and,

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting one or more of the primer extension products as an indication of one or more of the target nucleic acids.

3. (PREVIOUSLY PRESENTED) A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature, T_1, for denaturation of the strands of the target nucleic acids or their primer extension products,

(B) priming the denatured strands with a set of unmodified or modified primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2, and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3, provided that

when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same,

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, a hot start DNA polymerase and a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridisation.

4. (PREVIOUSLY PRESENTED) A method for the coamplification of two or more target nucleic acids having different sequence compositions and are present at comparable copy numbers whereas the maximum difference between the lowest and the highest copy number is 10 fold, said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequential steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature, T_1, for denaturation of the strands of the target nucleic acids or their primer extension products, and

(B) priming the denatured strands with a set of primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2, and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3, provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same, and

wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, a hot start DNA polymerase and optionally a sequence specific probe which binds within the primer binding regions and which generates a fluorescent signal after hybridisation, and,

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle, detecting one or more of the primer extension products as an indication of one or more of the target nucleic acids.

5. (PREVIOUSLY PRESENTED) A method according to one of claims 1 – 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate.

6. (PREVIOUSLY PRESENTED) A method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula:

$$H\text{-}(\text{-}O\text{-}R\text{-})_n\text{-}H$$

wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000.

7. (PREVIOUSLY PRESENTED) The method according to claim 6, characterized in that R may represent 1,2-ethylene, 1,3-propylene, 1,2-propylene, 2-hydroxy-1,3-propylene, 3-hydroxy-1,2-propylene, 1,4-butylene, 1,3-butylene, or 1,2-hexylene.

8. (PREVIOUSLY PRESENTED) The method according to claim 6, characterized in that the polyether is poly(ethylene glycol).

9. (PREVIOUSLY PRESENTED) The method according to claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 1000 to 2,000,000.

10. (PREVIOUSLY PRESENTED) The method according to Claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 to 500,000.

11. (PREVIOUSLY PRESENTED) The method according to Claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000.

12. (PREVIOUSLY PRESENTED) The method according to claim 5, characterized in that the volume exclusion reagent is a dextran.

13. (PREVIOUSLY PRESENTED) The method according to claim 12, characterized in that the dextran has a molecular weight in the range of 1000 to 2,000,000.

14. (PREVIOUSLY PRESENTED) The method according to Claim 12, characterized in that the dextran has a molecular weight in the range of 3000 to 500,000.

15. (PREVIOUSLY PRESENTED) The method according to Claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 to 60,000.

16. (PREVIOUSLY PRESENTED) The method according to claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-dihydroxypropyl acrylate).

17. (CURRENTLY AMENDED) ~~An amplification~~ A coamplification reaction composition for performing the method of any one of Claims 1-4, wherein said composition ~~which~~ is buffered to a pH of from about 7.5 to about 9, and wherein said composition comprises ~~comprising~~:
one or more sets of primers,
a thermostable hot-start[[-]] DNA polymerase,
a plurality of dNTP's, and
1 to 20 weight %, preferably from 1 to 15 weight % and most preferably from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent, and
optionally a probe.

18. (CURRENTLY AMENDED) The ~~amplification~~ coamplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 15 weight % of a nonionic, polymeric volume exclusion agent.

19. (CURRENTLY AMENDED) The ~~amplification~~ coamplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent.

20. (CURRENTLY AMENDED) A kit for the coamplification of two or more target nucleic acids according to the method of any one of Claims 1-4, comprising:
(a) ~~an amplification~~ a coamplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:
one or more sets of primers,
a thermostable hot-start DNA polymerase,
a plurality of dNTP's, and
1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a nonionic, polymeric volume exclusion agent, and
(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble substrate.

21. (CURRENTLY AMENDED) A self-contained test device for performing the coamplification method of any one of Claims 1-4, comprising, in separate compartments:
(a) ~~an amplification~~ a coamplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:
one or more sets of primers,
a thermostable hot-start DNA polymerase,
a plurality of dNTP's, and
1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a nonionic, polymeric volume exclusion agent, and,
(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble substrate, the compartments being connected in the test device so that the amplification reaction composition can be brought into contact with the capture reagent after amplification without opening the test device.

22. (CURRENTLY AMENDED) A kit for preparing ~~an amplification~~ a coamplification reaction composition according to claim 17 comprising:
at least one hot-start DNA polymerase, and
at least one polymeric exclusion reagent.